Voltage-dependent modulation of the neuronal nicotinic acetylcholine receptor-channel by cartap

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Abstract: Nicotinic acetylcholine receptor-channels (AChR) are believed to be the target site of cartap. In order to clarify the mechanisms of the cartap interaction with nicotinic AChRs, single-channel patch clamp experiments were performed using rat clonal phaeochromocytoma (PC12) cells. Cartap increased the short closures occurring during single-channel openings induced by acetylcholine (ACh). Thus, when co-applied with acetylcholine, cartap markedly shortened the open time associated with bursts. These effects were voltage-dependent, being more prominent at negative voltages. These results strongly suggest that cartap acts as an open channel blocker at the neuronal nicotinic AChR.

Keywords: cartap; insecticide; acetylcholine receptor; single-channel; ion channel; voltage dependency; patch clamp

1 INTRODUCTION

The nicotinic acetylcholine receptor (AChR) is reported to be the target of various chemical agents including general anesthetics and several natural and environmental toxins.[1–8] Cartap, *S*,*S*′-(2-dimethylaminotrimethylene)bis(thiocarbamate), is a synthetic analog of nereistoxin, 4-*N*,*N*-dimethylamino-1,2-dithiolane, derived from the marine annelid *Lumbriconereis heteropoda* Marenz, and has been shown by biochemical and electrophysiological studies to act on nicotinic AChRs.[9–16] Our own previous studies suggested that cartap might modify AChR single-channel kinetics, perhaps as an open channel blocker.[17,18]

We now report the results of additional single-channel patch clamp experiments further detailing the mode of action of cartap on nicotinic AChRs in rat clonal phaeochromocytoma (PC12) cells. Cartap increased the gaps or short closures occurring during the channel openings. Several single-channel kinetics, including the open time, the closed time and the burst duration were modified by cartap in a voltage-dependent manner. The data strongly suggest that cartap blocks the neuronal nicotinic AChR in the open state.

2 MATERIALS AND METHODS

2.1 Culture of PC12 cells

The PC12 cell line was kindly provided by Drs Edson X Albuquerque and Edna FR Pereira of the University of Maryland School of Medicine in Baltimore. Cells were cultured in Dulbecco's modified Eagles' medium containing fetal bovine serum (0.1 mg ml^{-1}, Sigma, St Louis, MO) at 36°C in air + carbon dioxide (90 + 10, by volume). For patch clamp experiments, cells were plated on glass coverslips coated with poly-L-lysine and cultured for two to seven days. PC12 cells expressing nicotinic AChRs did not require nerve growth factor treatment.

2.2 Cell-attached single-channel current recording

Single-channel currents were recorded using the cell-attached variation of the patch clamp technique at room temperature (22°C). Pipette electrodes were made from 0.8 mm (ID) borosilicate glass capillary tubes, coated by SigmaCote® (Sigma) to minimize background noise, and fire-polished. The electrodes had a resistance of 10–12 megohms when filled with standard pipette solution. The membrane was hyperpolarized to various potentials from a resting potential of $-55 \sim -60$ mV.

Currents were recorded using an Axopatch 200B amplifier filtered at 3 kHz, and stored at 88 kHz on a video cassette recorder via an analog-to-digital converter (VR 10B, Instrutech Corp, Elmont, NY). Current records were analyzed by the pClamp version 6.0 software (Axon Instruments). Only those

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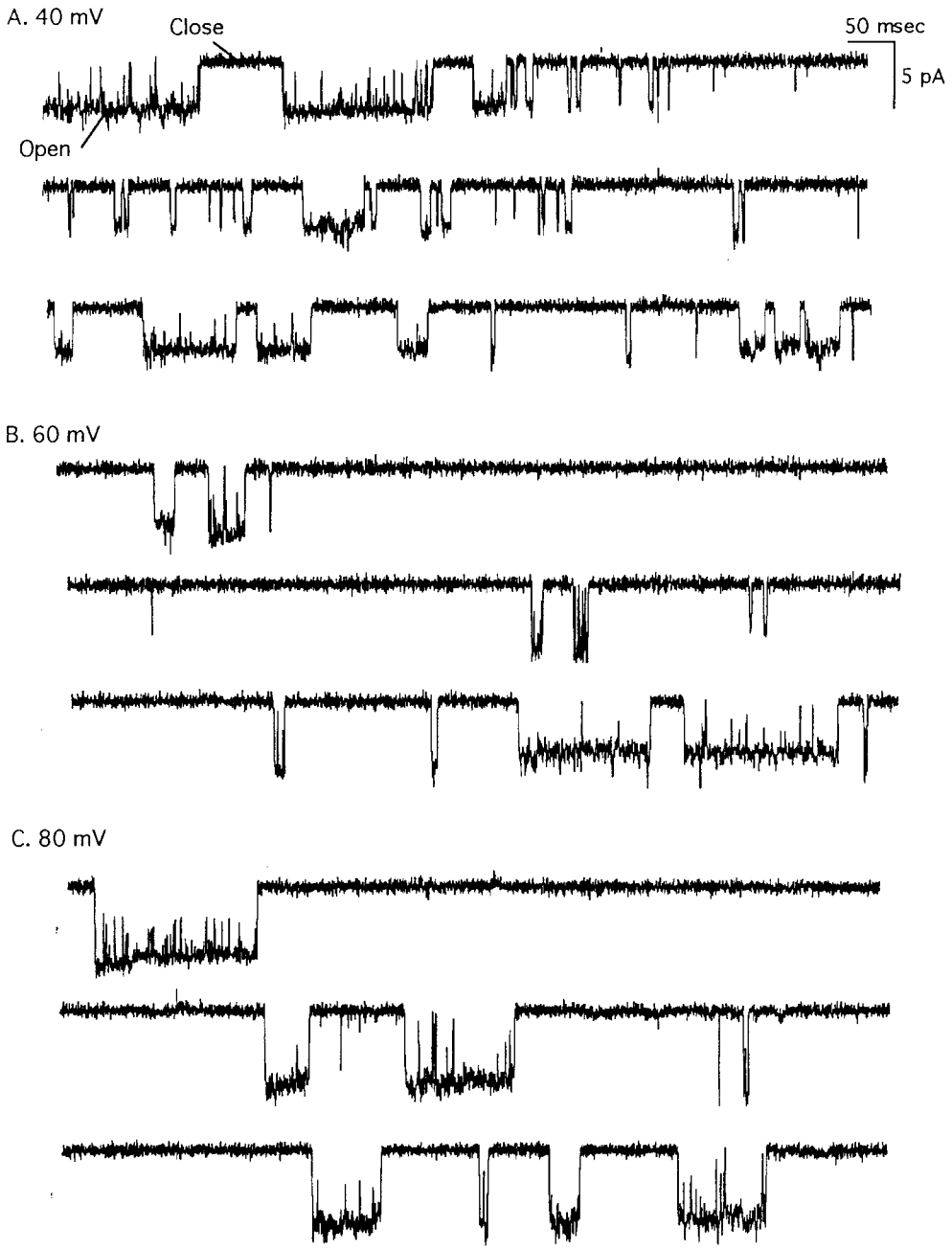

Figure 1. Single-channel currents generated by co-application of 10 μM ACh and 10 μM cartap to cell-attached membrane patches. Membrane patch clamped at a membrane potential A. 40 mV more positive, B. 60 mV more positive, C. 80 mV more positive than the resting potential.

events greater than 200 μs were considered in the analysis. Channel opening and closing were detected using the 50% threshold criterion of Colquhoun and Sigworth.[19] The interburst interval was also determined by the methods of Colquhoun and Sakmann.[20]

Data are expressed as the mean (±SD), where n represents the number of events used in estimating the values of current amplitude means, open times, closed times and burst durations.

2.3 Solutions

The external bath solution contained: NaCl(165 mM), KCl(5 mM), $CaCl_2$(2 mM) and *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid (HEPES; 5 mM). Tetrodotoxin (1 μM) was also added to eliminate sodium channel currents. The pH was adjusted to 7.3 with sodium hydroxide, and the osmolarity was adjusted to 330 mOsm by adding D-glucose.

2.4 Chemicals

Acetylcholine was dissolved in distilled water to make a stock solution of 10 mM. This stock solution was diluted with pipette solution to the final working concentration. Cartap was dissolved in dimethylsulfoxide (DMSO). The final DMSO concentrations were 3 ml litre^{-1} or less and had no effect on ACh-induced single-channel currents.

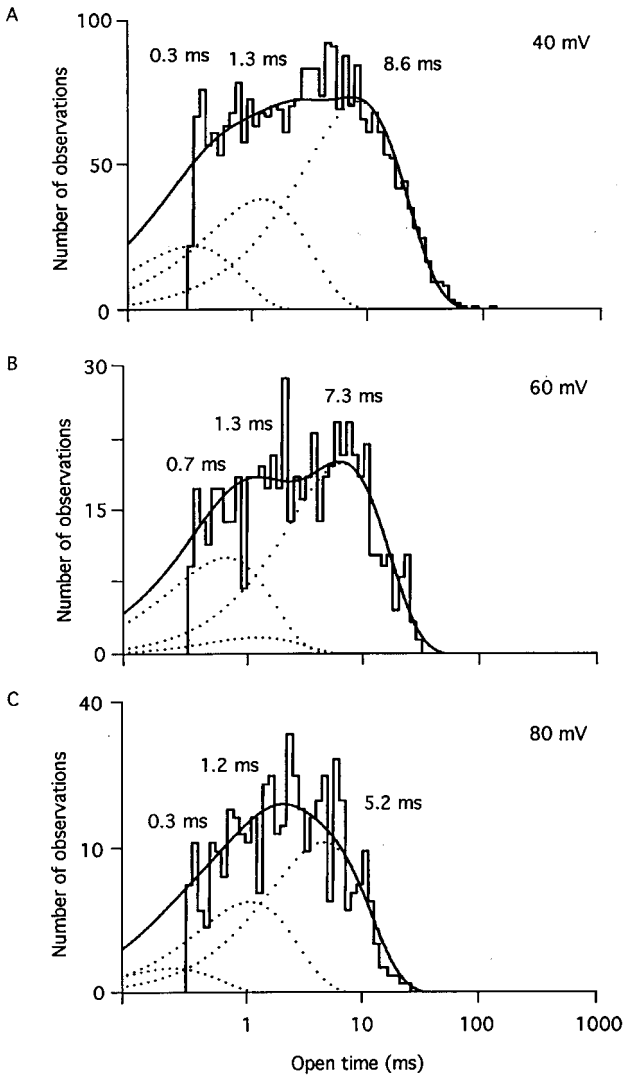

Figure 2. Open time distributions for main conductance currents induced by co-application of 10 μM ACh and 10 μM cartap. Membrane patch clamped at a membrane potential A. 40 mV more positive, B. 60 mV more positive, C. 80 mV more positive than the resting potential. The distributions are shown on a logarithmic time axis. The best fit of exponential functions is shown.

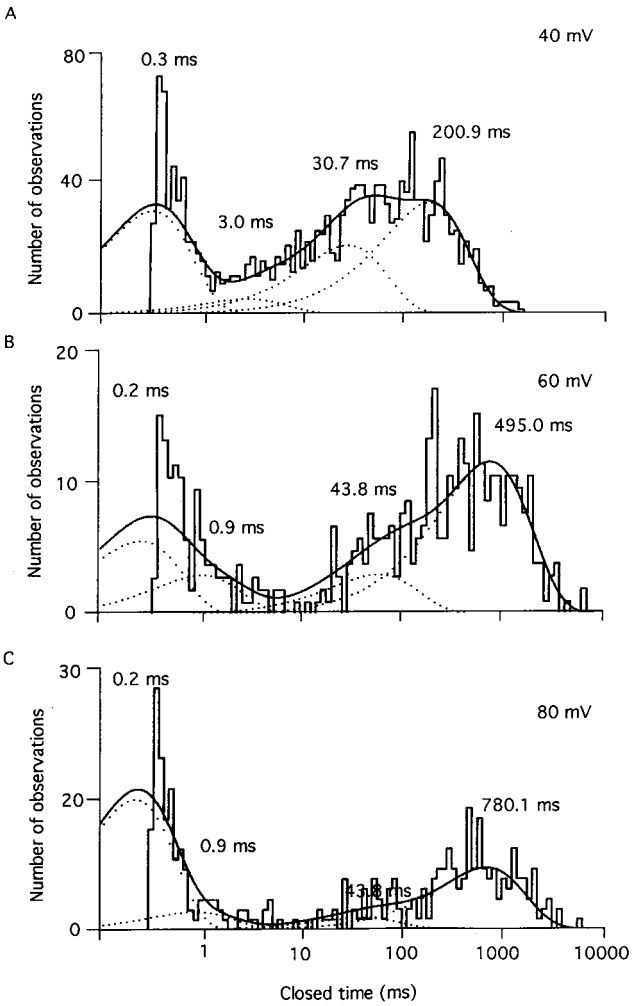

Figure 3. Closed time distributions for main conductance currents induced by co-application of 10 μM ACh and 10 μM cartap. Membrane patch clamped at a membrane potential A. 40 mV more positive, B. 60 mV more positive, C. 80 mV more positive than the resting potential. The distributions are shown on a logarithmic time axis. The best fit of exponential functions is shown.

3 RESULTS

Single-channel currents were recorded by the cell-attached patch clamp technique using a recording electrode containing 10 μM ACh with or without 10 μM cartap (Fig 1A). The membrane was hyperpolarized at various potentials below the resting potential. The resulting inward single-channel currents were generated by the activation of nicotinic AChRs.[6,7] It has been previously shown that multiple ACh channel conductances can be observed in PC12 cells.[7] Only the high-frequency conductances were analyzed in the present study.

When the membrane was hyperpolarized by 40 mV from the resting potential, a co-application of 10 μM ACh and 10 μM cartap evoked channel openings shorter than those of control. Current amplitude, however, was unchanged (Fig 1A). With a membrane hyperpolarization of 60 mV from the resting potential, current amplitude increased in proportion to the increased driving force on ionic flux. At this potential, cartap increased the openings and closings occurring within a burst to higher frequencies than observed at 40 mV (Fig 1B). When the clamp potential was increased to 80 mV, current amplitude increased further as did the intraburst opening and closing frequency (Fig 1C).

The channel open time distributions evoked by the application of 10 μM ACh and 10 μM cartap to cell-attached membrane patches clamped at various potentials are shown in Fig 2. The open time distribution at 40 mV clearly indicates multi-exponential components (Fig 2A). There are at least three components. These time constants and their relative proportions are 8.6 ms (54.6%), 1.3 ms (28.8%), and 0.3 ms (16.6%). The open time distribution of currents clamped at 60 mV also indicated multi-exponential components (Fig 2B). The slowest component had a time constant of 7.2 ms (62.2%),

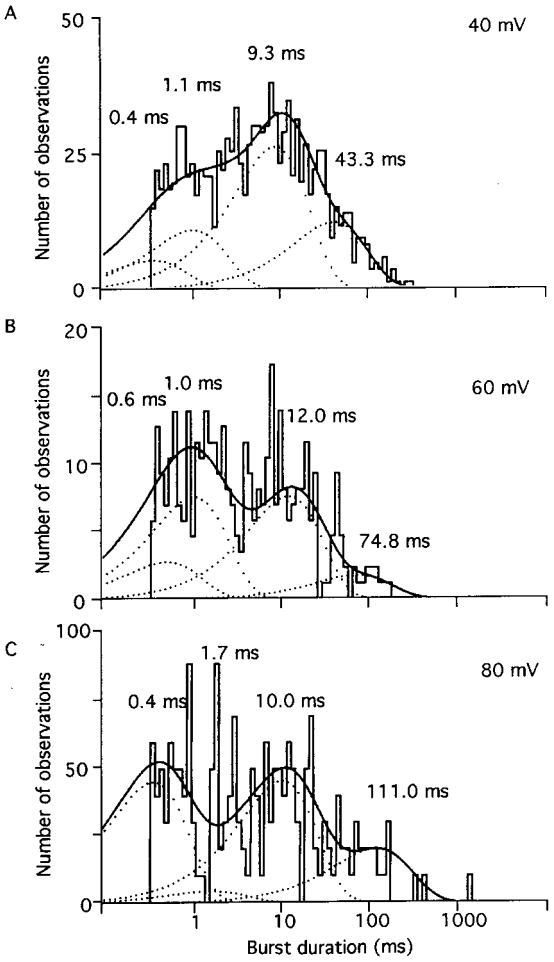

Figure 4. Distributions of burst duration for main conductance currents induced by co-application of 10 μM ACh and 10 μM cartap. Membrane patch clamped at a membrane potential A. 40 mV more positive B. 60 mV more positive, C. 80 mV more positive than the resting potential. The distributions are shown on a logarithmic time axis. The best fit of exponential functions is shown.

the intermediate component had a time constant of 1.4 ms (6.0%), and the fastest component had a time constant of 0.7 ms (31.8%). The open time distribution of currents clamped at 80 mV were also multi-exponential (Fig 2C), with a slow time constant of 5.2 ms (56.0%), an intermediate time constant of 1.2 ms (34.0%), and a fast time constant of 0.3 ms (10.0%). Therefore it appears that the slowest time constants were shortened by cartap in a voltage-dependent manner.

The distributions of single-channel closed times activated by 10 μM ACh in the presence of 10 μM cartap in cell-attached membranes at various potentials are shown in Fig 3. The closed time distribution of currents clamped at 40 mV indicated at least four exponential components (Fig 3A). The time constants and their relative percentages of the total histogram were 200.9 ms (37.4%), 30.7 ms (22.9%), 3.0 ms (5.0%), and 0.3 ms (34.7%). The closed time distribution of currents clamped at 60 mV also exhibited multi-exponential components (Fig 3B). The slowest component had a time constant of 495.0 ms (49.8%). The next two components had time constants of 43.8 ms (12.9%) and 0.9 ms (13.0%). The fastest component had a time constant of 0.2 ms (24.3%). Similarly, the closed time distribution of currents clamped at 80 mV also indicated multi-exponential components (Fig 3C). The slowest time constant was 780.1 ms (27.8%), the next slowest component had a time constant of 42.6 ms (5.2%), the next had a time constant of 0.9 ms (8.3%), and the fastest component had a time constant of 0.2 ms (58.7%). Thus, the slowest time constant was increased by cartap in a voltage-dependent manner.

The burst durations distributions induced by 10 μM ACh in the presence of 10 μM cartap at various potentials are shown in Fig 4. The distribution of burst durations of currents clamped at 40 mV was multi-exponential (Fig 4A) having at least four components. The time constants were 43.3 ms (22.3%), 9.3 ms (47.8%), 1.1 ms (20.0%), and 0.4 ms (9.9%). Burst duration distributions clamped at 60 mV also had four exponential components (Fig 4B). The time constants were 74.8 ms (10.5%), 12.0 ms (38.4%), 1.0 ms (36.2%), and 0.6 ms (14.9%). A four exponential distribution of burst durations clamped at 80 mV was also observed (Fig 4C). The time constants were 111.0 ms (17.4%), 10.0 ms (39.5%), 1.7 ms (3.8%), and 0.4 ms (39.3%). Once again, the slowest time constant was increased by cartap in a voltage-dependent manner.

4 DISCUSSION

Our previous study demonstrating that cartap increased short closures or gaps during channel opening without changing the single-channel conductance could explain the blocking effect of cartap on neuronal nicotinic AChR channels.[17,18] Open channel blockers are known to induce very short closures or gaps when the channel is open, producing a bursting channel activity in the single-channel record. These effects are both concentration- and voltage-dependent.[1]

Cartap similarly decreased the mean open time within the burst, an effect dependent on both concentration and voltage. This strongly suggests that cartap acts as an open channel blocker of neuronal nicotinic AChR in PC12 cells. In another preparation, using the nervous system of the American cockroach, we have shown that the blocking effect of cartap on the synaptic transmission is also voltage-dependent (Iwanaga *et al*, unpublished data).

It should be pointed out that cartap suppresses a neuronal type of nicotinic AChR in PC12 cells. Recent studies have been suggested that similar neuronal nicotinic AChRs play an important role in the activity of the central nervous system. The release of glutamate and GABA may be modified by Ca^{2+} ions entering through nicotinic ACh receptor channels in presynaptic membranes.[5] Similarly, the functioning of other ion channels, including those

activated by NMDA and GABA(A) receptors, may be modulated by Ca^{2+} entry through neuronal nicotinic AChRs involving a second messenger system. Thus, besides having a direct effect on neuronal nicotinic AChRs, cartap could indirectly modify other ion channels as well. Further studies will be necessary to address this issue.

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